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Stability-Indicating RP-HPLC Method for Determination of Ursodeoxycholic Acid in Tablet Dosage Form

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The aim of the present study is to develop and validate a novel, simple, selective and sensitive stability indicating reverse phase HPLC method for the determination of ursodeoxycholic acid (UDCA) in tablet dosage form after being subjected to different stress conditions, such as hydrolysis (0.1 N HCl and 0.1 N NaOH), oxidation (30 % H_2O_2), heat (80 ºC for 48 h) and photolysis (UV, 254 and 366 nm). The validation studies were carried out as per the International Conference on Harmonization (ICH) and United States Pharmacopoeia (USP) guidelines. An isocratic HPLC method was developed to separate ursodeoxycholic acid from the degradation products, using a BDS Hypersil C_8 column (thermo, 250 mm × 4.6 mm, 5 µ) with an isocratic mobile phase comprising of methanol, water and phosphoric acid (77:23:0.6 v/v).The flow rate was 1.0 mL/min and was carried out in refractive index detector. Retention time for ursodeoxycholic acid was about 3 min. A linear response was observed in the range of 240-360 µg/mL, (r^2 = 0.995) for the drug. The drug was decomposed in acid, base, 30 % H_2O_2 and in heat but was found to stable in photolytic stresses. The method was validated in terms of linearity, precision, accuracy, specificity, limit of detection and quantitation and robustness. The procedure was found to be specific, linear, precise (including intra and inter day precision), accurate and robust. Applicability of the method has been illustrated performing the assay of the drug in the tablet.

Key Words: HPLC, Stability indicating method, Validation, Ursodeoxycholic acid.

INTRODUCTION

Poorly soluble compounds tend to be eliminated from the GI tract before they have had opportunity to fully dissolve and be absorbed into the circulation. Ursodeoxycholic acid (UDCA) belongs to biopharmaceutical classification system (BCS) class II and hence it exhibits low aqueous solubility and high permeability. It is a white, odourless, crystalline powder with a bitter taste. Chemically it is 3α,7β-dihydroxy-5-cholan-24-oic acid (Fig. 1). It is a bile acid, a substance naturally produced by the body that is stored in the gall bladder. It works by decreasing the production of cholesterol and by dissolving the cholesterol in bile so that it cannot form stones. However, the low aqueous solubility and poor dissolution of this molecule in gastric fluid affects its rate of absorption, resulting in a low and variable oral bioavailability. It is used as a drug for the dissolution of cholesterol gallstones[1-3] because it reduces the cholesterol saturation of bile[4]. The use of UDCA for the treatment of other liver diseases, such as primary biliary cirrhosis, chronic hepatitis and biliary pains has been demonstrated[5-7]. However *in vivo* studies have shown that intestinal absorption and consequently the bioavailability of the drug are generally poor and erratic both among different subjects and within the same subject[8]. More than 50 % is lost in the stool[9] after a single oral dose of 300 mg.

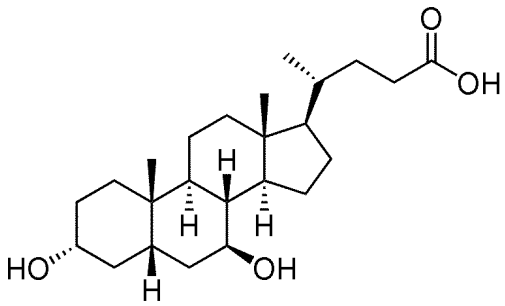

Fig. 1. Ursodeoxycholic acid (CAS number 128-13-2)

According to current good manufacturing practices (cGMP), all drugs must be tested with a stability-indicating assay method before release. The objective of the study is to develop and validate a simple and sensitive stability-indicating reverse phase high performance liquid chromatographic (RP-HPLC) assay method for the determination of ursodeoxycholic acid in tablet dosage form after forced degradation studies according to ICH and USP recommended test conditions.

EXPERIMENTAL

The raw drug ursodeoxycholic acid (UDCA) was gifted by Albert David Ltd, Kolkata, India and the tablet excipients like microcrocrystalline cellulose, povidone K-30, cross povidone, polyethylene glycol 6000, magnesium stearate, colloidal silicon dioxide, Eudragit L100, hydroxy propyl methyl cellulose and titanium dioxide were procured from Stadmed Pharmaceuticals Pvt. Ltd., Kolkata, India. All the reagents used were of analytical grade and were purchased from Merck. Methanol of HPLC grade was obtained from Merck (Darmstadt, Germany). All aqueous solutions including the buffer for the mobile phase were prepared with water (resistivity of 18.2 M ohm cm) collected from a Milli-Q gradient system of Millipore (Elix 3, Milli-Q A10 Academic).

Instrumentation and chromatographic conditions: The HPLC system was of a waters (USA), consisting of a solvent delivery pump (model No. 515), a refractive index detector of Waters (Model No. 2414) with empower 2 software for integration. Separation was achieved using a BDS Hypersil C_8 column (thermo, 250 mm × 4.6 mm, 5 µ). The isocratic mobile phase pumped at a flow rate of 1 mL/min consisted of methanol, water and phosphoric acid (77:23:0.6 v/v). The freshly prepared mobile phase was filtered through 0.45 µm filter (Millipore, Milford, MA and U.S.A) and degassed by sonication for 15 min. The injection volume was 25 µL and all the separations were performed at room temperature using refractive index detector set at temperature 40 ºC with sensitivity 64.

Preparation of stock and standard solution: The stock solution (1mg/mL) was prepared by weighing and dissolving 10 mg of UDCA into 10 mL of methanol. Aliquot of the standard stock solution of UDCA was prepared with mobile phase to get the required final concentrations.

Preparation of sample solution for assay: Twenty fixed dose combination tablets of UDCA were powdered and powder equivalent to 300 mg of UDCA was extracted into 100 mL of methanol by vortex mixing followed by ultrasoncation. It was then filtered through 0.45 µ filter and diluted with mobile phase to make 150 µg/mL of UDCA for analysis. The resulting solution was then injected into the column and chromatographed using the conditions mentioned above. The percent drug content was determined from the area of the peak using the regression equation obtained in the calibration experiments.

Method development: For analysis of UDCA in bulk drug and in the formulation, a variety of mobile phases were tried in the development of an HPLC method. Sensitivity, suitability for stability studies, time required for the analysis and ease of preparation was considered for selecting the mobile phase.

Method validation: The method was validated according to ICH[10-13] and USP guidelines[14]. The validation parameters addressed were linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantitation (LOQ) and robustness[15-17].

Linearity: For linearity, the test solutions were prepared by diluting primary stock solution (1 mg/mL) at nine concentration levels from 240-360 µg/mL. The solutions were injected in triplicate and three separate linearity curves were constructed. The slope and intercept were calculated.

Precision: Interday precision was performed after injecting six replicate of the drug solution in five different concentrations (240, 270, 300, 330 and 360 µg/mL).The same study is repeated on three different days to determine inter-day precision. The concentrations were calculated from the areas obtained and the results were expressed as percentage relative standard deviation (RSD %).

Accuracy: Accuracy was evaluated by fortifying reaction solution with three different concentrations (240, 270, 300, 330 and 360 µg/mL) of the drug. The recovery of the added drug was determined.

Specificity: The specificity of the method was established by injecting sample solutions of the drugs in presence of their degradation products and determining the peak purity.

Limit of detection (LOD) and limit of quantitation (LOQ): The LOD and LOQ of the drug were determined by using a signal to noise ratio of 3 and 10 respectively. The LOQ was verified by injecting 6 replicates at its concentration.

Robustness: The robustness of the developed method was established in different deliberately varied chromatographic conditions (flow rate, temperature, column from different manufacturers, solvents of different lots).

System suitability: The system suitability test was performed to check whether the complete testing system was suitable for the required application. A standard solution of 150 µg/mL was injected for six times. Peak area, retention time, theoretical plates and tailing factor were measured.

Stress studies: Tablet samples were prepared after grinding the fixed dose tablet and weighing of the powder equivalent to weight of the drugs. Powder equivalent to 300 mg of UDCA was weighed for each stress study separately and exposed to different experimental stress conditions described below.

Acid hydrolytic stress: The powder was dissolved in 10 mL of 0.1 N HCl and kept at 60 ºC for 3 h in water bath. After attaining the ambient temperature, the solution was neutralized by 0.1 N NaOH and volume was made upto 100 mL with water. 2 mL of the resulting solution was then diluted upto 10 mL with mobile phase to make a final solution containing 150 µg/mL of UDCA which was then centrifuged at 5000 rpm for 5 min and injected to the HPLC system.

Alkaline hydrolytic stress: The powder was dissolved in 10 mL of 0.1 N NaOH and kept at room temperature for 1 h. Then the solution was neutralized by 0.1 N HCl and volume was made upto 100 mL with water. The solution was then diluted and centrifuged as above and injected to HPLC system.

Oxidative stress: The powder was dissolved in 10 mL of 30 % H_2O_2 and kept at 60 ºC for 3 h in a water bath. After attaining ambient temperature, volume was made upto 100 mL with water and treated as same discussed above before injecting into the HPLC system.

Thermal stress: Powder was kept at 80 ºC for 48 h and the solution was prepared and diluted accordingly to achieve a final solution containing 150 µg/mL of UDCA which was then centrifuged and injected to the HPLC system.

UV Photolytic stress: The same amount of powder was exposed to UV short (254 nm) and UV long (366 nm) light for 48 h. Then the solution was prepared, diluted and centrifuged as above before injecting to HPLC system.

RESULTS AND DISCUSSION

Method development: After performing the chromatographic run with several solvent mixtures, the mobile phase consisting of methanol: water: phosphoric acid = 77:23:0.6 v/v was found to furnish sharp, well-defined peaks with good symmetry. It was observed that the developed chromatographic conditions provides better separation of ursodeoxycholic acid (UDCA) (3 min) as well as their degradation products in the chromatogram of forced degradation analysis of tablet samples. The typical representative chromatograms are shown in Fig. 2.

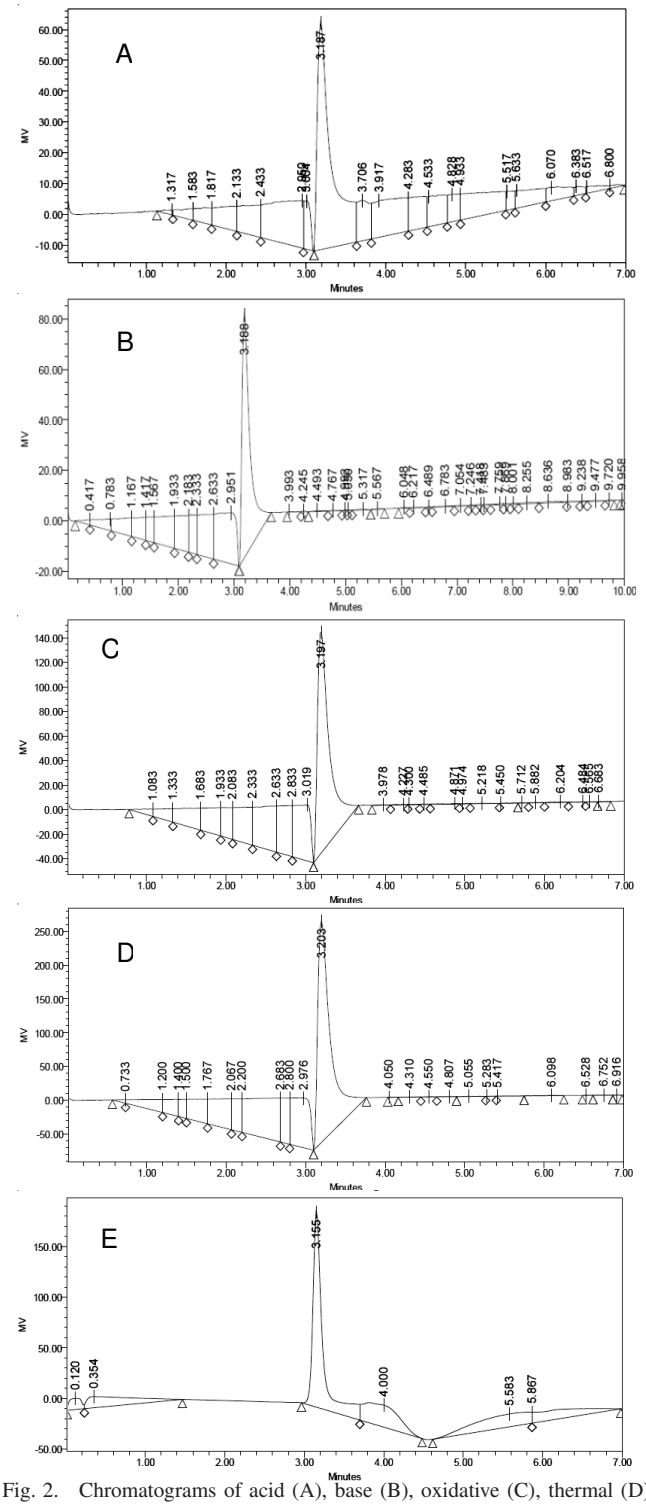

Fig. 2. Chromatograms of acid (A), base (B), oxidative (C), thermal (D) degradation study and assay in tablets (E)

Method validation

Linearity: A linear response was observed in the range of 240-360 µg/mL for the drug. The mean correlation coefficient (± RSD) for UDCA was 0.995 (Table-1) (Fig.3).

TABLE-1
SUMMARY OF VALIDATION AND SYSTEM SUITABILITY PARAMETERS

Parameters	Ursodeoxycholic acid
Linearity range (µg/mL)	240-360
Correlation coefficient (r^2)	0.995
LOD (µg/mL)	0.6
LOQ (µg/mL)	2
Accuracy (%)	100.0-100.4
Intra-day (n = 6) precision (RSD %)	0.097-0.448
Inter-day (n = 18) precision (RSD %)	0.073-0.346
Robustness	Robust
% RSD of peak area	1.117
Theoretical plates	325.0293
Tailing factor (asymmetry factor)	1.00

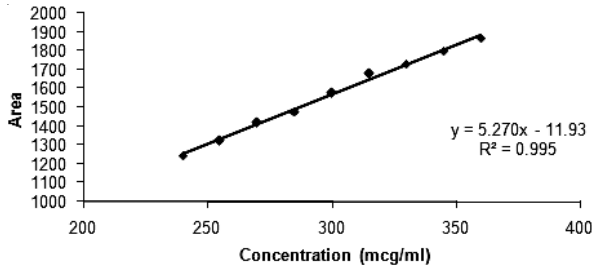

Fig. 3. Calibration curve of ursodeoxycholic acid

Precision: Data obtained for precision experiments are given in Table-1. The % RSD values for intra- and inter-day precision study was 0.144-0.332 % and 0.166-0.292 % respectively, which confirms that the method was sufficiently precise.

Accuracy: Difference between the peak areas obtained for fortified and unfortified solutions were used to calculate percentage recovery of the drugs. The recovery data indicates that excellent recoveries observed despite the presence of the degradation product of the drugs (Table-1).

Specificity: The specificity of the method can be justified from Fig. 2. where complete separation of the drugs from their degradation product was noticed. The average retention times for the degraded products were furnished in Fig. 2.

Limit of detection and limit of quantitation: The limit of detection (LOD) and the limit of quantitation (LOQ) were 0.6 and 2 µg/mL respectively. Low value of LOD and LOQ indicate the method is sensitive.

Robustness: Good separation of the drug and the degradation products is achieved after changing the flow rate from 0.8-1.0 mL/min, temperature from 35-40 ºC, column from different manufacturers and solvents of different lots.

System suitability: The experimental result shows that the parameters tested were within acceptable limit for % RSD of peak area, theoretical plates and tailing factor (Table-1).

Stress studies: No additional peak was found in the chromatogram of the sample undergone photolytic stresses (UV, 254 and 366 nm for 48 h). But additional peaks were observed in

the chromatogram of the sample undergone hydrolytic (0.1 N HCl, 60 ºC for 3 h and 0.1 N NaOH, room temperature for 1 h) and oxidative (30 % H_2O_2, 60 ºC for 3 h) stresses (Fig. 2). This indicates that the drug is stable in UV light but susceptible to degradation in acidic, alkaline hydrolysis, thermal and oxidation. The percentage degradation and percentage recovery data for stress degradation studies are summarized in Table-2. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective and stability indicating.

TABLE-2
SUMMARY OF DEGRADATION STUDIES
FOR URSODEOXYCHOLIC ACID

Stress condition	Time (h)	Degradation (%)	Recovery (%)
Acid, 0.1 N HCl, 60 ºC	3	23.741	76.259
Base, 0.1 N NaOH, RT*	1	22.168	77.832
30 % H_2O_2, 60 ºC	3	46.015	53.985
Thermal, 80 ºC	48	90.118	9.882
UV, 254 and 366 nm	48	-	-

*RT: Room temperature

Assay: Experimental results of the amount of UDCA in tablets expressed as percentage of label claim and were in good agreement suggesting no interference from the excipients of the tablet. The drug content was found to be 100-70 % for UDCA.

Conclusion

Literature survey reveals that there was no stability-indicating assay method for the determination of ursodeoxycholic acid (UDCA) in either bulk drug or in any pharmaceutical dosage form and hence the method developed in present investigation is a novel one. Different chromatographic methods have been described for the quantitative determination of UDCA, but those reported methods are not applicable to perform the stability indicating assay for the determination of UDCA in either bulk drugs or in fixed dose tablet. The chromatographic method developed is adequate for quantitation of UDCA in pharmaceutical dosage forms at different concentration levels. It is very simple, accurate and effective and provided no interference peaks for pharmaceutical excipients. Acceptable values of precision and accuracy have been obtained at all levels by this method regarding the guidelines for assay validation. The method uses simple mobile phase and is very beneficial for column life. The retention time of the drug is such that it distinguishes well from the degradant peaks. Applicability of the method has been illustrated performing the assay of fixed dose tablet. So the developed analytical method will be of immense help to the pharmaceutical industries for stability testing as well as routine quality control analysis of UDCA in bulk drug and pharmaceutical formulations.

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REFERENCES

1. G.D. Bell, R.H. Dowling, B.Whitney and D.J. Sutor, *Gut.*, **16**, 359 (1975).
2. I.A.D. Bouchier, *Ann. Res. Med.*, **31**, 59 (1980).
3. R.H. Dowling, *Clin. Gastroenterol.*, **12**, 125 (1983).
4. K. von Bergmann, M. Epple-Gutsfeld and O. Leiss, *Gastroenterology*, **87**, 136 (1984).
5. U. Leuschner, M. Leuschner and K. Hubner, *Gastroenterology*, **80**, 1208 (1981).
6. U. Leuschner and W. Kurtz, *Lancet*, **11**, 508 (1987).
7. A. Ward, R.N. Brogden, R.C. Reel, T.M. Speight and G.S. Avery, *Drugs*, **27**, 95 (1984).
8. M. Parquet, E.H. Metrman, A. Ralzman, J.C. Rambaud, N. Berthaux and R. Infante, *Eur. J. Clin. Invest.*, **15**, 171 (1985).
9. A. Stiehl, R. Raedsch and G. Rudolph, *Gastroenterology*, **98**, 424 (1990).
10. Text on Validation of Analytical Procedures, International Conference on Harmonization, September (1993).
11. Proceedings of the International Conference on Harmonization of Technical Requirements of Registration of Technical Requirements of Registration of Pharmaceuticals for Human Use (ICH Harmonized Triparite Guidelines, Validation of Analytical Procedures: Methodology, ICH-Q2B, Geneva (1996).
12. Q2 (R1) ICH guidelines, Validation of Analytical Procedures: Text and Methodology.
13. Proceedings of the International Conference on Harmonization of Technical Requirements of Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures, ICH-Q2A, Geneva (1995).
14. United States Pharmacopoeia 28, National Formulary 23, Validation of Compendial Methods. Rockville: U.S. Pharmacopoeial Convention Inc; 2748-51, (General Chapter 1225) (2005).
15. S. Baboota, *Acta Chromatogr.*, **18**, 116 (2007).
16. F.A. Chaibva and R.B. Walker, *J. Pharm. Biomed. Anal.*, **43**, 179 (2007).
17. P. Sengupta, A. Das and T.K. Pal, *Asian J. Chem.*, **22**, 6471 (2010).